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The global peripheral chemoreflex drive in patients with systemic sclerosis: a rebreathing and exercise study

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Abstract

Background: Exercise intolerance (EI) in systemic sclerosis (SSc) is difficult to manage by the clinician. The peripheral chemoreflex drive compensates for metabolic acidosis during exercise and may be related to EI.

Aim: To assess the global peripheral chemoreflex drive (GPCD) in patients with SSc at rest and during exercise.

Methods: Consecutively tested SSc patients (n=49) were evaluated by pulmonary function tests, carbon dioxide (CO₂) rebreathing studies and non-invasive cardiopulmonary exercise testing (CPET). Results of their CO₂ rebreathing tests were compared with those of controls (n=32). Respiratory compensation for metabolic acidosis during CPET was defined by the occurrence of a sharp increase in minute ventilation (VdotE) and the ventilatory equivalent for CO₂ (VdotE/VdotCO₂) at the end of the isocapnic buffer phase. Euoxic (eVHR) and hyperoxic (hVHR) ventilatory responses to hypercapnia were measured and its difference (eVHR-hVHR) was considered to reflect the GPCD.

Results: In 45 patients with SSc, CPET results showed respiratory compensation at the occurrence of metabolic acidosis. eVHR-hVHR in patients with diffuse cutaneous SSc (dcSSc) differed significantly from that in patients with limited cutaneous SSc (lcSSc) and from that in controls (0.47±0.38 (dcSSc) versus 0.90±0.77 (lcSSc) and 0.90±0.49 (controls) L/min/mmHg; p=0.04 and p=0.03, respectively).

Conclusions: Respiratory compensation for metabolic acidosis occurred in all patients. However, the GPCD was diminished in dcSSc patients, suggesting an altered control of breathing. Its assessment may help the clinician to better understand reported exercise intolerance and exertional dyspnea in dcSSc patients.
Background

Typically, progressive systemic sclerosis (SSc) may involve interstitial lung disease (ILD) and pulmonary hypertension (PH) (1). In some cases of progressive SSc, however, thoracic wall involvement may arise and manifest as an impairment in chest wall excursions caused by thickened thoracic skin, referred to as "sclerodermic chest wall" (2). The impedance of the respiratory system is influenced by lung and chest wall compliance and respiratory flow resistance (2;3). In progressive SSc, dyspnea may arise from an increased impedance of the respiratory system caused by ILD. In SSc, ILD or limited chest wall excursions due to a thickened thoracic skin is considered to cause this increased impedance. Consequently, ventilatory impairment as a result of restriction may occur, resulting in alveolar carbon dioxide (CO₂) retention and subsequently in hypercapnic respiratory failure (2;3). It has been postulated that hypercapnic respiratory failure and exercise intolerance (EI) may, in contrast, involve a gradual down-regulation in central and peripheral chemosensitivity, resulting in slightly chronic elevated arterial partial carbon dioxide pressures (PaCO₂) and reported dyspnea during exercise (2;3). Therefore respiratory failure and EI in SSc may include not only increased respiratory impedance (i.e. reduced respiratory compliance and/or increased flow resistance), but also a diminished peripheral chemoreflex drive. Moreover, an absent peripheral chemoreflex drive itself may induce an early onset of metabolic acidosis and therefore an exercise intolerance and reported exertional dyspnea (4;5).

The peripheral chemoreflex drive plays an important role in the control of breathing (4;5). It not only ensures oxygen homeostasis, but also helps maintain CO₂ levels at rest and during exercise (4-6). Activation by peripheral chemoreceptors has been implicated in ventilatory compensation for metabolic acidosis during exercise (4-6). This ventilatory compensation is reflected in a sharp increase in the ventilatory equivalent for CO₂ (VdotE/VdotCO₂) at the end of the isocapnic buffer phase and a decrease in end-tidal pCO₂ (6).

The pathophysiology of SSc is complex, involving immune activation and widespread vascular injury (7-9). Although SSc is primarily a microvascular disorder with perivascular cellular infiltrates that consist of macrophages, T cells and B cells, with a predominance of CD4+ T cells, macrovascular involvement has been reported as well (7-9). The carotid bodies, the site of the peripheral chemoreflex to oxygen, CO₂ and pH, contain a complex microvascular anatomy in a macrovascular environment. SSc-related inflammatory and fibrotic responses may cause a diminished peripheral chemoreflex, which may result into an increased susceptibility to EI and consequently reported dyspnea during exercise (4-6;10).
therefore hypothesized that the peripheral chemoreflex drive in normocapnic SSc patients is diminished at rest and during exercise. We obtained CO₂ rebreathing studies in SSc patients and healthy controls, and all SSc patients performed a cardiopulmonary exercise test (CPET).

Methods

Ethics

The local Medical Ethical Committee of the Leiden University Medical Center approved the protocol. Written informed consent was obtained from each participant prior to enrolment in the study.

Patients and healthy controls

We consecutively tested 49 SSc patients referred to an outpatient health care program. All patients were included in the study and underwent pulmonary function tests (PFTs), CO₂ rebreathing tests at rest and non-invasive incremental CPET on a bicycle according to Wasserman (6). No patients were excluded. All tests were done in one day or on two consecutive days between April 2010 and January 2012. Patients were classified as having limited cutaneous sclerosis (lcSSc) or diffuse cutaneous systemic sclerosis (dcSSc) according to the LeRoy criteria (11). All healthy controls performed the CO₂ rebreathing tests at rest only; PFTs were expected to be normal.

Euoxic (eVHR) and hyperoxic (hVHR) ventilatory response to hypercapnia at rest

CO₂ rebreathing tests were first obtained in all subjects. To assess the global peripheral chemoreflex drive (GPCD), we assessed the ventilatory response to hypercapnia under both euoxic (eVHR) and hyperoxic (hVHR) conditions as previously described (12). Briefly, under hyperoxia, the peripheral chemoreflex drive is considered to be suppressed for at least 30 minutes (13;14). The central chemoreflex drive to hypercapnia can therefore best be assessed for several minutes during hyperoxia. When the ventilatory response to hypercapnia under euoxia is measured, the total chemoreflex drive consists of both central and peripheral chemoreflex drives (15-17). These drives are then assumed to be additive (14;17). Thus, when the inspiratory fraction of oxygen in room air is kept constant at a level of 21%, the global
contribution of the peripheral chemoreceptor to total ventilatory response to hypercapnia can be evaluated by calculating eVHR minus hVHR (eVHR-hVHR) (12;14-17).

In SSc, increased respiratory impedance may be present as a result of ILD, thoracic wall involvement or increased flow resistance (2;3;18). However, because we measured eVHR and hVHR in a single SSc patient on the same day with a limited time interval, we did not consider the respiratory impedance to influence the peripheral chemoreflex loop gain (eVHR-hVHR). As a result, the GPCD may be compared between SSc patients and controls, irrespective of increased respiratory impedance.

**Non-invasive CPET**

Symptom-limited, noninvasive incremental CPET (without arterial blood gas sampling) was next performed under physician supervision in all SSc patients (19). To evaluate the presence of a peripheral chemoreflex drive during exercise, we monitored minute ventilation (VdotE) and VdotE/VdotCO₂ continuously by breath-by-breath analysis. The anaerobic threshold was determined by use of the lowest ventilatory equivalent for oxygen (VdotE/VdotO₂) or the V-slope method whenever appropriate (19). Normally, when exercise continues until the occurrence of metabolic acidosis (i.e. at the end of the isocapnic buffer phase), a sharp increase in VdotE/VdotCO₂ provides ventilatory compensation which reflects an active peripheral chemoreflex drive (Figure 6.1). In all CPET results, this response was scored qualitatively as being either present or absent (6;10). Since this response is expected in all healthy controls, they did not undergo CPET.

**Statistical analysis**

Statistical analysis was performed with the SPSS 20.0 package (SPSS, Inc., Chicago, IL, USA). Continuous variables are expressed as the mean value ± standard deviation. P-values <0.05 were considered significant. Categorical data are presented as frequencies and percentages. Statistical comparisons were performed by using the Student’s t-test for continuous variables and the chi-square test for binary variables.
Results

Study population characteristics

Our 49 SSc patients were extensively characterized (Table 6.1). LcSSc patients (n=20) differed significantly from patients with dcSSc (n=29) with respect to disease duration, duration of skin disease, modified Rodnan skin score, onset of Raynaud phenomenon, and current and previous treatment.

Pulmonary function tests

DcSSc patients differed significantly from lcSSc patients in predicted forced vital capacity (FVC%; p=0.034) and total lung capacity, helium dilution method (TLC-He%; p=0.034) (Table 6.2). Furthermore, gas transfer (DLCO single breath method) was impaired in both groups, although it was not significantly different. These parameters may indicate the presence of increased respiratory impedance and therefore ILD, PH or both.
### Table 6.1  Clinical characteristics of 49 SSc patients and 32 healthy controls

<table>
<thead>
<tr>
<th></th>
<th>lcSSc n=20</th>
<th>dcSSc n=29</th>
<th>Controls n=32</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female, no. (%)</td>
<td>18 (90)</td>
<td>18 (62)</td>
<td>16 (50)</td>
<td>0.35</td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>57.1 (8.8)</td>
<td>49.1 (12.2)</td>
<td>45.4 (15.8)</td>
<td>0.65</td>
</tr>
<tr>
<td>Ethnicity, no.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>19</td>
<td>22</td>
<td>32</td>
<td>-</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>African</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Disease duration, years, median (IQR)</td>
<td>10.1 (8.1)</td>
<td>4.7 (4.5)</td>
<td>-</td>
<td>0.009</td>
</tr>
<tr>
<td>Skin duration, years, median (IQR)</td>
<td>11.7 (9.3)</td>
<td>6.1 (6.4)</td>
<td>-</td>
<td>0.022</td>
</tr>
<tr>
<td>Onset of Raynaud phenomenon, months, median (IQR)</td>
<td>14.8 (11.2)</td>
<td>8.5 (8.9)</td>
<td>-</td>
<td>0.039</td>
</tr>
<tr>
<td>MRSS (0–51), mean (SD)</td>
<td>3.9 (4.1)</td>
<td>7.8 (6.8)</td>
<td>-</td>
<td>0.003</td>
</tr>
<tr>
<td>Current treatment, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0 (0)</td>
<td>6 (19)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Prednisone</td>
<td>1 (0.04)</td>
<td>4 (13)</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Azathioprine</td>
<td>0 (0)</td>
<td>4 (13)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Previous treatment, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>0 (0)</td>
<td>14 (45)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Stem cell transplantation</td>
<td>0 (0)</td>
<td>10 (32)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>2 (8)</td>
<td>15 (48)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Prednisone</td>
<td>2 (8)</td>
<td>11 (35)</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

SSc, systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; dcSSc, diffuse cutaneous systemic sclerosis; SD, standard deviation; IQR, interquartile range; MRSS, modified Rodnan skin score. * Chi square, Student t-test, Mann Whitney U test or Fisher exact test where appropriate between limited and diffuse SSc.

### Table 6.2  Pulmonary functions tests and ventilatory responses to hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>lcSSc n=20</th>
<th>dcSSc n=29</th>
<th>Controls n=32</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (% pred)</td>
<td>101 (21)</td>
<td>84 (20)</td>
<td>-</td>
<td>0.034*</td>
</tr>
<tr>
<td>DLCOcSB (% pred)</td>
<td>63 (19)</td>
<td>57 (15)</td>
<td>-</td>
<td>0.19*</td>
</tr>
<tr>
<td>TLC-He (% pred)</td>
<td>91 (21)</td>
<td>80 (16)</td>
<td>-</td>
<td>0.034*</td>
</tr>
<tr>
<td>eVHR</td>
<td>2.59 (1.27)</td>
<td>1.87 (0.75)</td>
<td>3.08 (0.75)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>hVHR</td>
<td>1.70 (0.98)</td>
<td>1.41 (0.62)</td>
<td>2.46 (0.91)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>eVHR-hVHR</td>
<td>0.90 (0.77)</td>
<td>0.47 (0.38)</td>
<td>0.90 (0.49)</td>
<td>0.04**</td>
</tr>
</tbody>
</table>

* P-value expressed for Student t-test between lcSSc and dcSSc.  P-value expressed for Student t-test between dcSSc and controls.  P-value expressed for Student t-test between lcSSc and dcSSc. lcSSc, limited cutaneous systemic sclerosis; dcSSc, diffuse cutaneous systemic sclerosis; FVC, forced vital capacity (L); % pred, percentage predicted; DLCOcSB, carbon monoxide gas transfer factor corrected for hemoglobin, single breath method (mmol/min/kPa); TLC-He, total lung capacity, helium-dilution method (L); eVHR, euoxic ventilatory response to hypercapnia (L/min/mmHg); hVHR, hyperoxic ventilatory response to hypercapnia (L/min/mmHg); eVHR-hVHR, global peripheral chemoreflex drive (L/min/mmHg).
Euoxic and hyperoxic ventilatory response to hypercapnia

In healthy controls, the mean eVHR was 3.08±0.75 L/min/mmHg and differed significantly from that in dcSSc patients (1.87±0.75 L/min/mmHg; p<0.001). Similarly, the mean hVHR in controls differed significantly from that in dcSSc patients (2.46±0.91 versus 1.41±0.62 L/min/mmHg; p<0.001), but not from that in lcSSc patients (1.70±0.98 L/min/mmHg; p=0.11).

Global peripheral chemoreflex drive (eVHR-hVHR) in dcSSc patients was diminished and differed significantly from that in lcSSc patients (0.47±0.38 in dcSSc versus 0.90±0.77 in lcSSc; p=0.04) and from that in healthy controls (0.47±0.38 in dcSSc versus 0.90±0.49 L/min/mmHg in healthy controls; p=0.03). Figure 6.2 presents the mean (eVHR-hVHR) response in all subjects.

Furthermore, in 14 of 29 (48%) dcSSc patients, previous treatment consisted of autologous hematopoietic stem cell transplantation, which had a significant impact on the modified Rodnan skin score and on Raynaud phenomenon. In 15 dcSSc patients who were not treated with stem cells, the GPCD was significantly diminished compared with 14 stem-cell-treated dcSSc patients (0.27±0.23 and 0.64±0.42 L/min/mmHg, respectively, p=0.008).

Non-invasive CPET

In total, 45 SSc patients (20 lcSSc, 25 dcSSc) reached metabolic acidosis during incremental exercise (end of isocapnic buffer phase). Four SSc patients (all dcSSc) discontinued their exercise before reaching anaerobic threshold and reported exertional dyspnea. In all CPET results for these 45 SSc patients, ventilatory compensation to metabolic acidosis occurred
by means of a sharp increase in $V_{\text{dot}E}$ and $V_{\text{dot}E}/V_{\text{dot}CO_2}$ (i.e. Figure 6.1, data not shown) (19). Within these 45 SSc patients, peak aerobic capacity ($V_{\text{dot}O_2}^{\text{peak}}$), a parameter of exercise tolerance, did not differ between lcSSc and dcSSc patients (data not shown).

**Discussion**

In progressive SSc, the global peripheral chemoreflex drive may be altered. Our results show the presence of an active peripheral chemoreflex drive during maximal exercise in all patients with SSc, as indicated by ventilatory compensation for metabolic acidosis. However, the global peripheral chemoreflex drive, as measured by the difference in eVHR and hVHR, was diminished in our dcSSc patients compared with that in healthy controls.

This is the first study on the hypercapnic ventilatory response to evaluate the peripheral chemoreflex drive in patients with SSc. In the present study, global assessment of CO$_2$ responsiveness was used to characterize different populations. We used room air in a rebreathing bag and kept the inspired fraction of oxygen during the euoxic rebreathing test constant at a level of 21% (12). However, some considerations may apply to the methods used in the present study. First, age, sex, status of menstrual phase and ethnicity all affect the ventilatory response to CO$_2$ (20). In our study, age and sex were not significantly different between SSc patients and healthy subjects. Furthermore, the ventilatory response did not significantly differ between healthy male and female SSc patients in the eVHR and hVHR tests (data not shown). Therefore, taking these results together, we believe that these issues did not contribute significantly to differences in ventilatory responses. Second, some dcSSc patients were previously treated with high-dose cyclophosphamide and autologous hematopoietic stem cell transplantation. Such treatment was not given to patients with lcSSc and may have contributed to the variability in ventilatory responses.

We used the concept of testing the GPCD by subtracting ventilatory responses to different oxygen supplies, as previously suggested by Duffin (17). In mammals, the carotid bodies are responsible for 95% of the ventilatory response to hypoxemia (21) and 30% of the response to arterial hypercapnia. In patients with resected bilateral carotid bodies, a 20–40% decrease in ventilatory response to euoxic hypercapnia is observed (10) Under euoxia, the peripheral chemoreflex drive is less active than when measured under hypoxia and considered to be 50–80% of total peripheral chemoreceptor sensitivity (10;15;16;20). In a previous study, we evaluated the peripheral chemoreflex drive in paraganglioma patients by subtracting
the hyperoxic from the euoxic ventilatory response to CO₂ (11). Furthermore, by using different levels of oxygen it was possible to determine the quantitative contribution of the chemoreceptors to ventilation at different levels of peripheral chemoreceptor stimulation (16;22). Consequently, the GPCD to CO₂ could be estimated from the difference in the euoxic and hyperoxic slopes (11;15-17). We therefore designated peripheral chemoreflex sensitivity under euoxia minus the hyperoxic ventilatory response (eVHR-hVHR) as the global peripheral chemoreflex drive.

In the setting of ILD or PH, minute ventilation may be strongly influenced by increased respiratory impedance (i.e. reduced respiratory compliance and/or increased flow resistance). In our study, lcSSc and dcSSc patients differed significantly in global peripheral chemoreflex function. Moreover, since both eVHR and hVHR are measured in the same patient and within a limited time period, respiratory impedance is considered not to influence the peripheral chemoreflex loop gain (eVHR-hVHR). Therefore, a difference in the GPCD between lcSSc and dcSSc patients may be derived from our rebreathing studies.

In addition to its measurement during rebreathing studies, the peripheral chemoreflex function can be assessed during exercise (6;10). When the exercise work rate is high enough to produce metabolic acidosis, the increase in ventilatory response is only – and strongly – mediated by the peripheral chemoreceptors (6). Thus, in the setting of an absent carotid body function, respiratory compensation for metabolic acidosis does not occur (6;10). In all of our SSc patients, including dcSSc patients, a ventilatory compensatory response at the end of the isocapnic buffer phase occurred as a result of the presence of a peripheral chemoreflex drive. However, quantitatively, as our results from the rebreathing studies indicate, a diminished ventilatory response is present in dcSSc patients compared with that in healthy controls, suggesting an altered GPCD in the absence of metabolic acidosis.

Our results may be explained by two mechanisms. First, SSc is considered to be an inflammatory disease (8;9). Anti-inflammatory treatment by autologous stem cell transplantation may have reduced the level of inflammation in carotid bodies, as indicated by our results in dcSSc patients. In dcSSc patients treated with autologous hematopoietic stem cell transplantation, a significant difference was present in the GPCD compared with that in dcSSc patients without such treatment. Therefore, this drive may have been altered positively by stem cell transplantation, suggesting an impact on carotid body vascularization and consequently its function. Second, widespread atherosclerosis is present not only in lcSSc patients, but also in dcSSc patients, and may involve the carotid arteries and bodies (8;9).
In subjects affected by widespread atherosclerosis, carotid body parenchyma may show degenerative changes; we speculate that atrophy may influence carotid body function (i.e. peripheral chemoreceptors) (7-9). We did not, however, measure carotid artery intima thickness. Furthermore, serum lipid spectrum was not significantly different between lcSSc and dcSSc patients.

Conclusions

In summary, our results show that in all SSc patients, ventilatory compensation for metabolic acidosis occurs as a result of an active peripheral chemoreflex drive. The global peripheral chemoreflex drive was, however, diminished in dcSSc patients compared with that in healthy controls, suggesting an altered chemoreflex control of breathing in these patients. This may help the clinician to better understand reported exercise intolerance and exertional dyspnea in dcSSc patients.
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